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Prolongation of murine thyroid allografts by interleukin 2 (DAB486)-toxin and RS-61443*

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Abstract. We evaluated the efficacy of interleukin 2 (DAB486)-toxin (IL-2-diphtheria toxin fusion protein; IL-2-toxin) in combination with RS-61443 to prolong murine thyroid allograft survival. B10.BR thyroid allografts were transplanted beneath the renal subcapsule in recipient (C57BL/10 mice and graft survival determined 21 days later. Treatment with IL-2-toxin (25 μg/day for 14 days) was unable to prolong graft survival significantly. RS-61443 treatment (21 days) achieved significant graft prolongation only at doses of 300 mg/kg daily or greater. When both drugs were used in combination (IL-2-toxin, 25 μg/day for 14 days RS-61443 200 mg/kg daily for 21 days), statistically significant ($P < 0.0001$) graft prolongation was obtained. Our results suggest that IL-2-toxin in combination with subtherapeutic RS-61443 levels significantly prolongs murine thyroid allograft survival. IL-2-toxin and RS-61443, because of their unique and complementary mechanisms, hold promise for more selective immunosuppression.

Key words: Immunosuppression – Interleukin 2 – RS-61443

RS-61443, a morpholinoethyl ester of mycophenolic acid (MPA), has been shown to be effective in prolonging canine kidney allograft [12] and murine islet allograft [5] survival. In vitro studies indicate not only inhibition of T-cell proliferation and cytotoxic T-cell generation, but also of B-cell proliferation and antibody secretion [2, 4].

Strom and colleagues have used recombinant DNA methodologies to replace genetically the eukaryotic cell receptor binding domain of diphtheria toxin with sequences encoding interleukin 2 (IL-2) [9, 10, 15]. Entry of IL-2 (DAB486)-toxin (IL-2-toxin) into the cell is via the high affinity IL-2 receptor [1]. IL-2-toxin treatment has been shown to produce marked immunosuppression of the murine delayed type hypersensitivity (DTH) response [8], be cytotoxic for activated human T helper and B cells [3], prolong murine islet allograft survival [11], and suppress an autoimmune response [7].

In this study we have determined the efficacy of IL-2-toxin and RS-61443 individually and in combination to prolong murine thyroid allograft survival. IL-2-toxin was not effective in prolonging allograft survival. Low dose RS-61443 therapy was marginally able to prolong graft survival. In contrast, combination therapy consisting of IL-2-toxin and low dose RS-61443 was effective in prolonging allograft survival.

Materials and methods

Mice. The Jackson Laboratory, Bar Harbor, provided B10.BR and C57BL/10 mice. All animals were maintained according to guidelines prepared by the Committee on Care and Use of Laboratory Animal Resources, National Research Council (DHEW publication no. 78–23, revised 1978).

Thyroid harvest and transplantation. Thyroid glands were removed from donor B10.BR mice, placed in minimal essential medium, and transplanted as previously described [6]. Thyroid graft function was determined 21 days posttranspslant. Briefly, recipient mice were injected with 0.5 μCi carrier-free $Na^{125}I$ in saline (i. p.). Then 24 h later, the kidneys were removed and placed in saline buffered formalin. Incorporated counts were compared with background (counts incorporated into the nongrafted, right kidney), with a ratio ≥ 4.0 considered to be a functional, viable graft. Graft nonfunction due to rejection was confirmed by histology.

Histology. H & E stained sections (6 μ) were prepared. Histological scoring was determined by a blinded observer ($n = 3$): 0, no lymphocytic infiltrte; 1, small focal infiltrates; 2, moderate cellular infiltrates; 3, heavy cellular infiltrates; 4, heavy cellular infiltrates with graft destruction and necrosis.

Immunosuppression. RS-61443 was supplied in powdered form by Syntex (USA), Palo Alto. A suspension of Rs-61443 (20 mg/ml) in a carboxymethyl cellulose vehicle was prepared and stored at 4 °C. IL-2 toxin was provided by Seragen, Hopkinton, in lyophilized form and stored at −80 °C. Prior to administration, IL-2-toxin was reconstituted with TRIS buffered saline containing 0.1 % bovine serum albumin (BSA), aliquoted, and frozen (−20 °C) until use.

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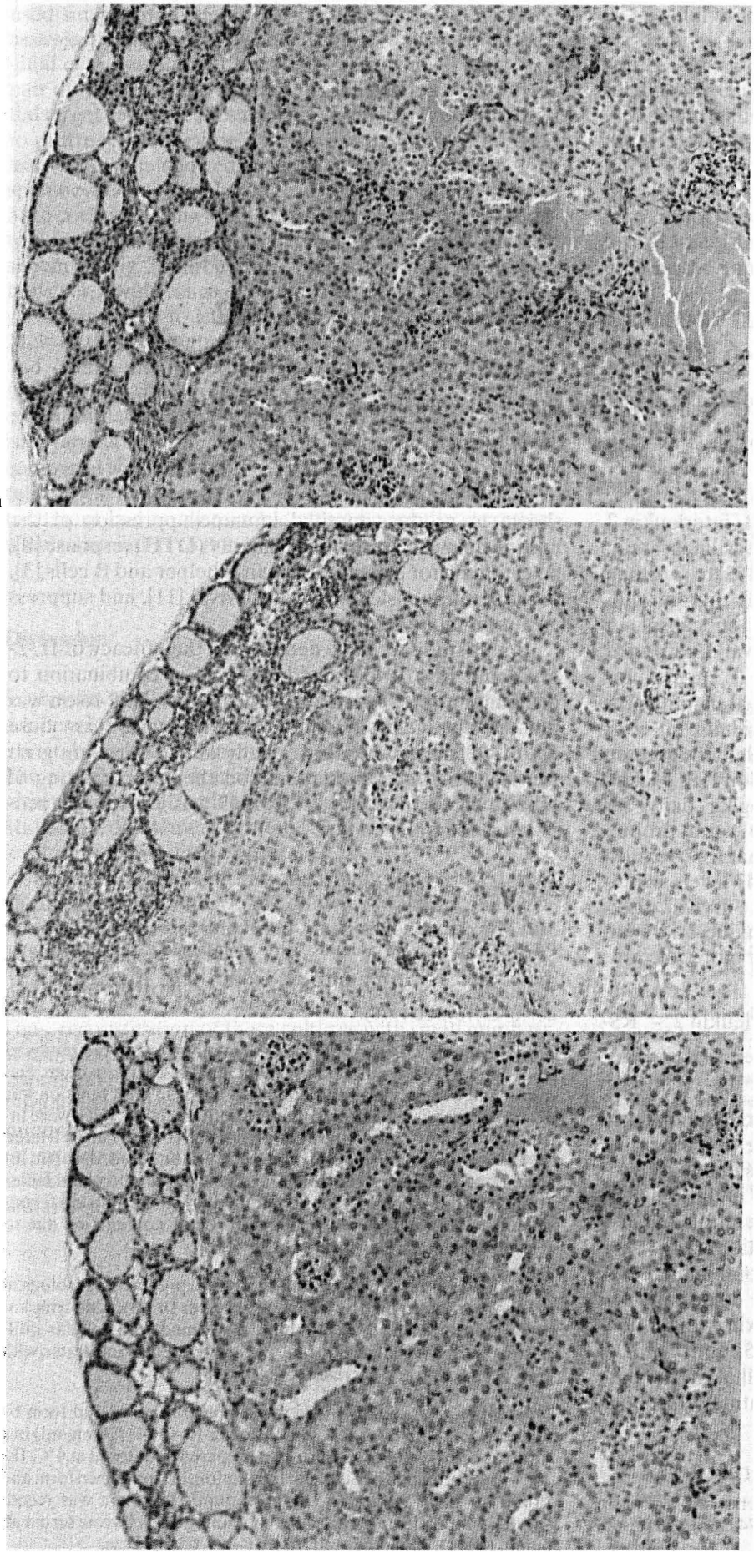

Fig. 1a–c. Combination IL-2-toxin and low dose RS-61443 therapy significantly improves murine thyroid allograft survival. **a** HVE of BIO.BR thyroid transplanted to BIO.Bl recipients; mean histological score 0.5. **b** HVE of C57BL/10 thyroid transplanted to BIO.BR receiving low dose (200 mg/kg) RS-61443 monotherapy; mean histological score 3.0. **c** HVE of C57BL/10 thyroid transplanted to BIO.BR receiving combination IL-2-toxin and RS-61443 therapy; mean histological score 1.5

Table 1. Combination therapy with RS-61443 and interleukin 2 (DAB486)-toxin prolongs murine thyroid allograft survival

Group	Recipient	Survival	Treatment[a]	Median[b] ^{125}I ratio	*P* value vs group 1	Histological score
1	B10.BR	21/22	–	18.8 ± 5.8		1
2	B10.BR	21/21	IL-2-toxin	12.5 ± 5.4	0.266	2
3	B10.BR	11/12	RS-61443	18.1 ± 5.1	0.666	1
4	B10.BR	7/7	RS-61443 plus IL-2-toxin	42.5 ± 11.7	0.272	2
5	C57BL/10	0/30	–	1.0 ± 0.09	0.0001	4
6	C57BL/10	9/34	IL-2-toxin	1.0 ± 1.01	0.0001	3
7	C57BL/10	20/31	RS-61443	6.9 ± 1.5	0.0001	4
8	C57BL/10	19/19	RS-61443 plus IL-2-toxin	25.2 ± 5.4	0.142	2

[a] Recipient animals were dosed with RS-61443 (200 mg/kg daily p.o.) for 21 days and/or IL-2-toxin for 14 days (25 μg/day s.c.)
[b] Graft function was determined at 21 days by ^{125}I incorporation. The counts perminute (CPM) incorporated into the left, grafted kidney were compared with the CPM incorporated into the right, nongrafted kidney. A ratio ≥ 4.0 was considered a viable graft

Recipient mice were dosed with RS-61443 by oral gavage beginning on the day before transplantation and continuing until the conclusion of the experiment at day 21. IL-2-toxyin was administered at 25 μg/day subcutaneously for 14 days beginning on the day of transplantation.

Statistical analysis. Median graft incorporation ratios were compared by the Mann-Whitney test.

Results and discussion

RS-61443 significantly improved graft survival at doses of 300 mg/kg daily or greater (data not shown). However, a significant weight loss was noted in recipients, most likely due to the gastrointestinal toxicity [12]. This dose is considerably greater than that reported for muring islet allografts [5]. The difference could be due to the fact that islet allografts are less immunogenic than thyroid grafts or to the strain combination used. At lower RS-61443 doses (200 mg/kg daily; Table 1), graft prolongation was achieved in 64% of recipients. Histological examination showed ongoing graft rejection and significant levels of lymphocyte infiltration (Fig. 1). This observation is also reflected in the median ^{125}I uptake ratio obtained for these grafts, indicating significant graft destruction and loss of functional viability (Table 1; 6.9 vs. 18.8 in controls). Significant weight loss did not occur at this dose.

IL-2-toxin alone failed to prolong thyroid allograft survival (Table 1). Again, this is in contrast to the results obtained with murine cardiac allografts. This difference may reflect immunogenicity differences between vascularized and nonvascularized grafts. The half life of IL-2-toxin in vivo is approximately 4.5 min (unpublished data). In a nonvascularized graft, sufficient amounts of IL-2-toxin may not be able to reach the graft to prevent rejection. In addition, activation of the T cell following IL-2 binding to its receptor requires approximately 18 h, while cell death following internalization of the diphtheria portion of the molecule requires 24 h [4–15]. Sufficient killing of activated T cells prior to the release of newly synthesized IL-2 may not occur, thus allowing newly synthesized IL-2 to compete effectively with IL-2-toxin [13, 14].

Eugui et al. [2] have shown that lymphocytes in the presence of RS-61443 express IL-2 receptors on their surface but are prevented from further differentiation and proliferation. Thus, activated T cells in the presence of RS-61443 are a ready target for IL-2-toxin therapy which requires the delivery of minimal amounts of toxin to the cytoplasm of the cell [13]. IL-2-toxin (25 μg/day) in combination with low dose RS-61443 (200 mg/kg daily) significantly prolonged thyroid allograft survival at 21 days (Table 1). When the median ^{125}I uptake ratios were compared, combination therapy resulted in significant improvement ($P > 0.0001$) and were comparable with those obtained in the synergic nontreated control group (Table 1). These results were confirmed by histology (Table 1; Fig. 1).

In this paper, we have demonstrated the immunosuppressive potential of IL-2-toxin and low dose RS-61443 combination therapy for prolonged murine thyroid allograft survival. The unique properties of these agents are complementary and together may provide a potent immunosuppressive therapy.

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